

## 寄生于龙眼裳卷蛾的微孢子虫一新种 (微孢子虫门, 布雷孢虫科)

万永继<sup>1, 2\*</sup> 刘仁华<sup>2</sup> 沈佐锐<sup>1</sup>

1. 中国农业大学昆虫学系 北京 100094

2. 西南农业大学蚕学与生物技术学院 重庆北碚 400716

**摘 要** 本种于 2000 年从四川阆中林木害虫龙眼裳卷蛾幼虫体内发现分离。孢子卵圆形 ( $3.1\mu\text{m} \pm 0.3\mu\text{m} \times 1.6\mu\text{m} \pm 0.2\mu\text{m}$ ), 孢子发育中呈现出八孢子囊孢子和二孢子母细胞类型, 为变形孢虫属的典型特征, 分别形成单核孢子和双核孢子, 极丝圈数分别为 7~9 圈和 10~12 圈; 从宿主、大小、极丝、发育等的差异检索表明: 为微孢子虫门变形孢虫属 *Vairimorpha* Pilly, 1976 1 新种, 定为裳卷蛾变形孢虫 *Vairimorpha ceraces* sp. nov.。

**关键词** 微孢子虫门, 双单倍期纲, 少双单倍期目, 布雷孢虫科, 变形孢虫属, 新种。

**中图分类号** Q59.115.92

龙眼裳卷蛾 *Cerace stipatana* Walker, 1863 为鳞翅目害虫, 危害龙眼、樟树、荔枝、梧桐、杨树和槐树等, 在我国主要分布于广东、浙江、福建、江西、四川、云南, 每年发生 4~5 代。1985~2000 年在四川曾数次发生龙眼裳卷蛾爆发性危害大叶樟、杨树和梧桐等。作者等从龙眼裳卷蛾虫体中检测到一种微孢子虫, 并对该微孢子虫做了系统研究, 生物学研究结果: 隶属布雷孢虫科, 变形孢虫属, 命名为裳卷蛾变形孢虫 *Vairimorpha ceraces* sp. nov.。模式标本保存在西南农业大学农业部重点实验室蚕桑病理病虫害研究室。

### 1 材料与方法

调查龙眼裳卷蛾幼虫自然感染率, 从病态幼虫中分离、纯化微孢子虫, 试验时将微孢子虫孢子液接种二龄幼虫, 置于培养箱中分 20℃和 26℃两个不同饲养区饲养。1) 观察病征, 调查寄生组织; 2) 中肠组织涂片 Giemsa 染色, 光镜观察发育过程和发育特征; 3) 测量孢囊及孢子大小, 测量统计模式:  $\text{Mean} \pm \text{SD}$  (Min Max),  $N=50$ ; 4) 患病中肠及肌肉组织做超薄切片, 在透射电子显微镜下研究微孢子虫的超微结构及微孢子虫与寄主的界面特征。

### 2 新种描述

裳卷蛾变形孢虫, 新种 *Vairimorpha ceraces* sp. nov. (图 1~10)

**寄主、寄生部位及病症:** 寄主龙眼裳卷蛾 *Cerace stipatana* Walker, 寄生中肠组织及中肠肌肉、

脂肪体和体壁表皮细胞。幼虫感染后在全身体壁上出现不规则黑斑点, 严重者身体明显缩短而死亡。

**模式产地:** 四川省阆中市。

**自然感染率:** 2000 年调查幼虫自然感染率约 25% (75/300)。

**孢子形态和大小:** 孢子卵圆形, 一端钝圆, 一端稍尖; 孢子的长径为  $3.1 \pm 0.3\mu\text{m}$  (2.5~3.5  $\mu\text{m}$ ), 宽径  $1.6 \pm 0.2\mu\text{m}$  (1.5~1.9  $\mu\text{m}$ ), 多数为 8 个孢子积聚状态, 也有二孢子特征 (图 6~7)。

**孢子超微结构:** 孢子为双核或单核。二孢子母细胞发育类型产生双核孢子, 极丝 10~12 圈 (图 9~10), 八孢囊体孢子为单核, 极丝 7~9 圈 (图 8)。孢壁 (spore wall) 清晰可见外壁 (EX) 和内壁 (EN), 孢子前端为极帽 (AD), 后端有后极泡 (PV) (图 10)

**生活史和发育特征** 发育周期 6~10 d, 生活史经过裂殖生殖期 (Merogony) 和孢子形成期 (Sporogony)。裂殖体 (meront) 球形, 具有强折光性, 大小开差 (图 1), 大型裂殖体向母孢子转化时, 折光性降低, 形成母孢子 (图 2); 进入孢子形成期母孢子 (sporont) 的染色性增强, 在光学显微镜下明显可见核的分裂, 八孢囊体的核分裂数从 1、2、4 至 8 个 (图 3~5), 然后细胞质分割形成 8 个单核孢子 (图 7), 在电镜下可观察到外围形成了一层孢囊膜 (图 8); 而小型裂殖体出现二分裂, 形成双核孢子 (图 5~6); 孢子的发育形态随饲养温度的不同而出现变异, 在 20℃的环境下为孢囊型产孢 (图 7), 26℃高温区时出现孢囊型产孢和二孢子母

国家自然科学基金 (30271006) 和重庆市应用基础 (02-7333) 项目资助。

\* 电话: 023-68251228; 023-68251380; 010-62733015; E-mail: Wanyj@swau.edu.cn

收稿日期: 2004-10-20, 修订日期: 2005-01-12。

细胞产孢同时存在的现象（图 6），这种孢子形成方式既有八孢囊体（Octospores）也有二孢子母细胞（bisporoblate）的生活史发育特征，属于变形孢虫属的典型特征。

微孢子虫与寄主细胞的界面特征：二孢子母细胞及孢子形成过程中没有膜的结构，直接与寄主细胞质接触（图 9），但孢囊型产孢体外围有一薄膜（图 8），此膜在孢子形成后期有时退化消失。

3 比较与讨论

与报道的变形孢属微孢子虫比较，本研究应用电镜技术首次在八孢囊型产孢体外观察到了孢囊薄膜，它为一柔性单层膜，推测它可能是抵御宿主的免疫反应胁迫微孢子虫自身合成的，明显不同于内网虫属微孢子虫 *Endoreticulatus* sp. （万永继等，1995）的孢囊膜，为来自于宿主细胞内质网的双层

膜。

变形孢虫属是由 Pilly, B. M. （1976）建立和描述的，模式种为寄生粘虫的纳卡变形孢虫 *Vairimorpha necatrix* (Kramer, 1965)，迄今该属已记录 7 个种，16 个未确定种和分离物。7 个种的数据显示，孢子的平均长径均大于 4 μm，宽径大于 2 μm，极丝圈数在 10~ 24 圈的范围内；其他 16 个未确定种和分离物虽然来自不同寄主，如棉铃虫 *Heliothis armigera*、切夜蛾 *Euxoa auxiliaris*、舞毒蛾 *Lymantria dispar* 等，但孢子大小和极丝圈数与 7 个种的数据相似，因此未确定种。本种的生活史发育形态学特征符合该属特征，但孢子大小和极丝圈数明显不同于上述种类（表 1）；卷蛾科裳卷蛾属有 4 种昆虫，寄生微孢子虫为新的记录；鉴于上述区别，确认本种为 1 新种。

表 1 裳卷蛾变形孢虫与同属种类的特征比较

Table 1. Characteristic comparison of *Vairimorpha ceraces* sp. nov., with all species/isolates of genus *Vairimorpha*.

微孢子虫种类 Microsporidia species	宿主 Type host and related hosts search	特征差异 Characteristic difference
纳卡变形孢虫（模式种） <i>Vairimorpha necatrix</i> (Kramer, 1965) (Type specis)	粘虫 <i>Pseudaletia unipuncta</i>	孢子的平均长度和宽度均超过 4 μm 和 2 μm，极丝圈数的范围为 10~ 24 圈 The every species all spores mean length and mean width are more than 4 μm and 2 μm. The polar filament coils number is in 10~24 range
谷螟变形孢虫 <i>V. plodiae</i> (Kellen and Lindegren, 1968)	印度谷螟 <i>Plodia interpunctella</i>	
异翅孢变形孢虫 <i>V. heterosporum</i> (Kellen, 1969)	异翅亚目昆虫 Heteroptera	
粉斑螟变形孢虫 <i>V. ephestiae</i> (Mattes, 1927)	地中海粉斑螟 <i>Ephestia kuehniella</i>	
瘤虻变形孢虫 <i>V. hybomitrae</i> (Levchenko and Issi, 1973)	瘤虻属昆虫 <i>Hybomitra</i>	
红火蚁变形孢虫 <i>V. invictae</i> (Jouvenaz and Ellis, 1986)	外来红火蚁 <i>Solenopsis invictae</i>	孢子大小为 3.1 μm±0.3 μm×1.6 μm±0.2 μm，极丝 7~ 12 圈 Spore size is 3.1 μm±0.3 μm×1.6 μm±0.2 μm; the polar filament numbers: 7~12 range
天蚕蛾变形孢虫 <i>V. antheraeae</i> (Yefimenko, 1987)	天蚕 <i>Antheraea yamamai</i>	
裳卷蛾变形孢虫，新种 <i>Vairimorpha ceraces</i> sp. nov.	龙眼裳卷蛾 <i>Cerace stipatan a</i>	

1. 变形孢虫属另有 16 个未确定种和分离物，来自不同寄主，但其孢子大小和极丝圈数与 7 个种的数据相似，没有相关寄主为龙眼裳卷蛾的记录。（There is other 16 *Vairimorpha* spp. or Isdates from the different hosts and similar to above seven species in the spores size and polar filament coils number, No have a record with host *Cerace stipatan a* by search)
2. 相关主要参考文献列在论文后。（Related key reference at the end the text)

致谢 美国华盛顿大学系统生物学研究所 M. Pan 博士提供部分文献，中国农业大学昆虫学系杨定教授

给予论文建议和指导，高灵旺博士协作图片整理；特此感谢。

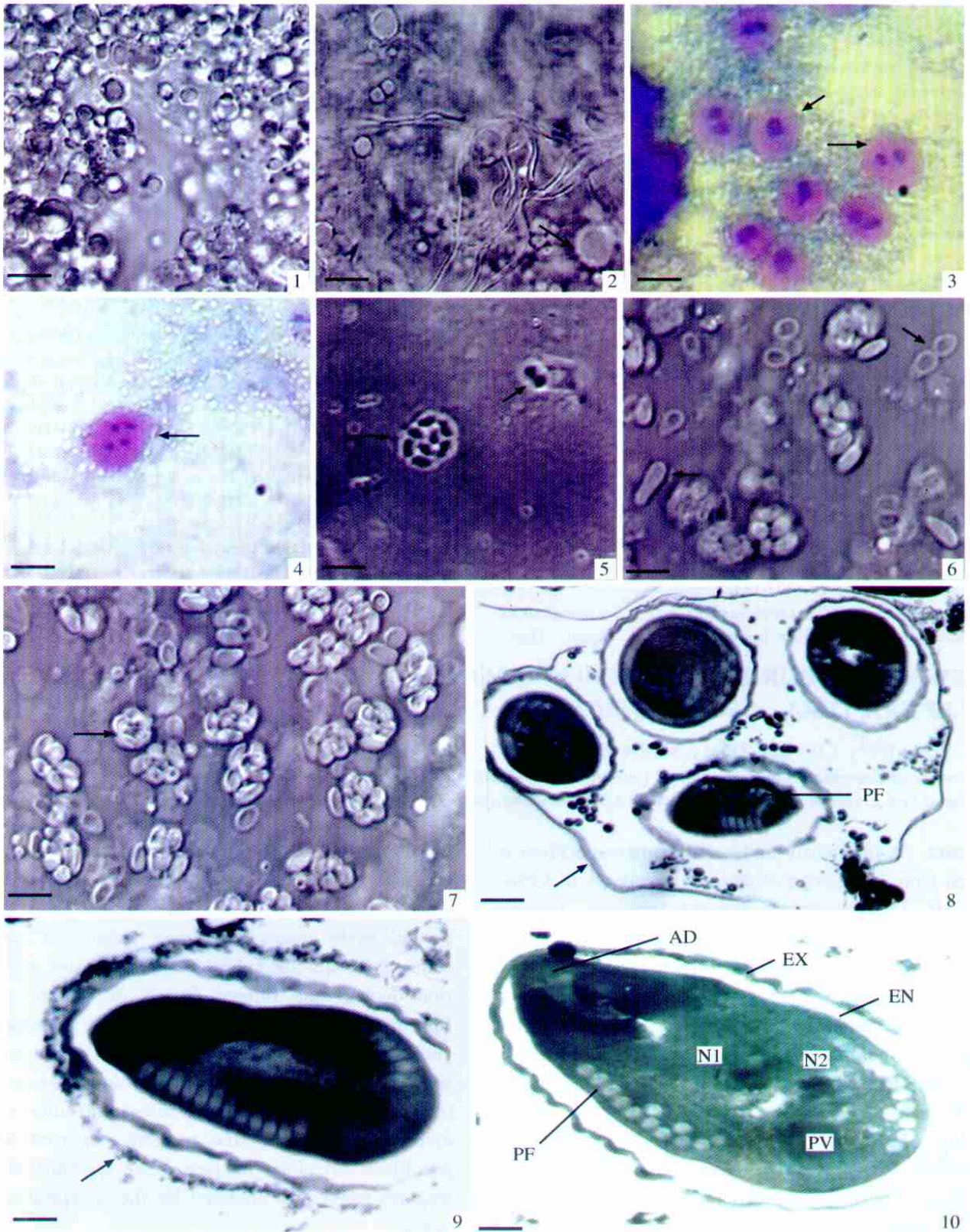


图 1~10 裳卷蛾变形孢虫的发育形态和超微结构

Figs 1-10. Developmental micrographs and fine structures of the microsporidium, *Vairimorpha ceraces* sp. nov. under light microscope and electron microscope.

1. 裂殖体 (meronts) 2. 裂殖体进入孢子发生期, 形成母孢子 (meront beginning transition to sporulation) 3~4. 母孢子染色性增强, 可见核的分裂和增加 (sporonts nucleus is developing and dividing) 5. 八孢子母细胞和二孢子母细胞 (sporoblasts with octospores form and bisporoblasts) 6. 26°C 条件二孢子母细胞及成熟孢子的发生 (bisporoblasts produced two mature binucleate spores in 26°C environment) 7. 20°C 条件八孢子母细胞及成熟孢子的产生 (octosporoblasts produced eight uninucleate spores in 20°C environment) 8. 形成八孢子囊膜内含短极丝单核孢子 (sporophorous membrane and its enclosed short polar filament spores with uninucleus) 9. 双核孢子形成时直接与寄主细胞质界面 (showing binucleate spore forming and host cytoplasm are face to face) 10. 孢子超微结构 (showing ultrastructure of spore) AD 极帽 (anchoring disc), EX 孢子外壁 (exospore), EN 孢子内壁 (endospore), PF 极丝 (polar filament), PV 后极泡 (polar vesicle), N1, N2 核 (binucleus) 比例尺 (scale bars): 1~7= 5μm, 8= 0.5μm, 9~10= 0.2μm



## REFERENCES (参考文献)

- Clyde, B. M. and Wayne, M. B. 1994. An ultrastructural study of the Epispontal inclusions produced during octosporogony by five Species/Isolates of *Vairimorpha* (Microspore: Microsporidia). *J. Invertebr. Pathol.*, 63: 197-206.
- Jouvenaz, D. P. and Eillis, E. A. 1986. *Vairimorpha invictae* n. sp. (Microspore: Microsporidia), a parasite of the red imported fire ant, *Solenopsis invictae* Buren (Hymenoptera: Formicidae). *J. Protozool.*, 33: 457-461.
- Larson, R. 1986. Ultrastructure, function and classification of Microsporidia. *Prog. Protistol.*, 1: 325-390.
- Larson, J. I. R. 1999. Identification of Microsporidia. *Acta Protozool.*, 38: 161-197.
- Leellen, F. S. and Joseph, V. M. 1998. Timing an early sporulation sequence of Microsporidia in the genus *Vairimorpha* (Microspore: Burenellidae). *J. Invertebr. Pathol.*, 72: 323-329.
- Malone, L. A. and Canning, E. U. 1982. Fine structure of *Vairimorpha plodid* (microspore: Burenellidae), a pathogen of *Plodia interpunctella* (Lepidoptera, pyritidae) and infectivity of the dimorphic spores. *Protistol.*, 18: 503-516.
- Pilley, B. M. 1976. A new genus, *Vairimorpha* (Protozoa: Microsporidia), for *Nosema necatrix* Kramer 1965: Pathogenicity and life cycle in *Spodopatera exempta* (Lepidoptera: Noctuidae). *J. Invertebr. Pathol.*, 28: 177-183.
- Pekkarinen, M. et al. 2002. Oviplesiphora gen. n., a new genus for *Pleiosiphora mirandellae*-like microsporidia. *Dis. Aquat. Org.*, 48: 133-142.
- Sprague, V. 1977a. Classification and phylogeny of the Microsporidia. In: Comparative Pathobiology, Vol. 2. Plenum, New York pp. 1-30.
- Sprague, V. 1977b. Annotated list of species of Microsporidia. In: Comparative Pathobiology, Vol. 2. Plenum, New York pp. 51-212.
- Steett, D. A. and Briggs, J. D. 1982. Variation in spore polypeptides from four species of *Vairimorpha*. *Biol., Syst., Ecol.*, 10: 161-165.
- Sedlacek, J. D. et al. 1985. Effects of temperature and dosage on *Vairimorpha* sp. 696 spore morphometric spore yield, and tissue specificity in *Heliothis virescens*. *J. Invertebr. Pathol.*, 48: 320-324.
- Sprague, V. et al. 1992. Taxonomy of phylum microspore. *Critical Reviews in Microbiology*, 18: 285-395.
- Wen, J-Z, Li, S-P and Sun, GX 1992. New record of *Vairimorpha necatrix* (Microspore: Burenellidae). *Acta Zootaxonomica Sinica*, 17 (2): 116-117. [问锦曾, 李社平, 孙传信, 1992. 纳卡变形孢子虫中国新记录. 动物分类学报, 17 (2): 116-117]
- Wen, J Z 1999. Microspore, Protozoology. Science Press, Beijing. 393-399. [问锦曾, 1999. 原生动物学, 微孢子虫门. 北京: 科学出版社. 393-399]
- Wan, Y-J, Zhang, L and Chen, Z P 1995. Study of a pathogenic microsporidia SCM<sub>7</sub> (*Endoreticulatus* sp.) isolated from the larva of silkworm *Bombyx mori*. *Acta Sericologica Siniica*, 21: 168-173. [万永继, 张琳, 陈祖佩, 1995. 家蚕病原性微孢子虫 SCM<sub>7</sub> (*Endoreticulatus* sp.) 的分离和研究. 蚕业科学, 168-173]

## A NEW MICROSPORIDIUM (MICROSPOREDIA, BURENELLIDAE) FROM THE INSECT OF LEPIDOPTERA, CERACE STIPATANA (WALKER)

WAN Yongji<sup>1,2</sup>, LIU Renhua<sup>2</sup>, SHEN Zuorui<sup>1</sup>

1. Department of Entomology, China Agricultural University, Beijing 100094, China

2. College of Sericulture and Biotechnology, Southwest Agricultural University, Chongqing, Beibei 400716, China

**Abstract** A previously undescribed microsporidium is isolated from the *Cerace stipatana* (Walker) in October 2000, in Langzhong, Sichuan Province, China. We describe this microsporidium as a new species of *Vairimorpha*, and name it as *Vairimorpha ceraces* sp. nov. According to taxonomy system (Sprague, 1992), it belongs to Phylum Microspora, Class Dihalophasea, Order meiodialophasea and Family Burenellidae.

**Type Host.** *Cerace stipatana* Walker (Lepidoptera, Tortricidae).

**Site of Infection.** Infection to midgut epithelial and muscle cells, fat body, derma cell.

**Type Locality.** Langzhong, Sichuan Province (32°N, 105°E), China.

**Prevalence of infection.** Seventy-five of three hundred larvae, *Cerace stipatana* examined were infected (25%).

**Diagnosis.** Spore is oval in shape and  $3.1 \mu\text{m} \pm 0.3 \mu\text{m}$  ( $2.5-3.5 \mu\text{m}$ )  $\times$   $1.6 \mu\text{m} \pm 0.2 \mu\text{m}$  ( $1.5-1.9 \mu\text{m}$ ) in size. Binucleate spore with 10 to 12 coils of polar filaments, and uninucleate spore with 7 to 9 coils.

**Key words** Microspora, Dihalophasea, Meiodialophasea, Burenellidae, *Vairimorpha*, new species.

The spore size and polar filament coil numbers are smaller than other species of *Vairimorpha*. Developmental cycle passes merogony stage and sporogony stage. Meronts are spherical in shape and multiplication depend the binary fission of meront nucleus. Sporonts are binucleate and fusiform. Sporogony includes not only octosporoblast form with uninucleate spore but also bisporos form with binucleate spore. Interface feature between the microsporidium and host cytoplasm showing that meronts, sporont and bisporoblasts are face to face. But sporonts form octospores when it is enclosed by the sporophorous membrane.

**Type specimens.** Syntypes with spores on slide (No. cq20001012) and spores put into small glass bottle were deposited in the Southwest Agricultural University, Key Sericulture Laboratory of the Agricultural Ministry, China.